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# Methods and Costs of Producing *Nomuraea rileyi* Conidiospores



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## CONTENTS


| | Page |
|---|---|
| Abstract | 1 |
| Introduction | 1 |
| Spore production | 1 |
| Method | 1 |
| Yield | 5 |
| Cost | 6 |
| Laboratory bioassays | 6 |
| Spore counts | 6 |
| Biological activity | 6 |
| Microbial contamination | 7 |
| Discussion | 7 |
| References | 7 |


## ILLUSTRATIONS


| Fig. | | |
|---|---|---|
| 1. | Weighing Sabouraud's maltose agar powder medium | 2 |
| 2. | Agar powder distributed into carboys for heating and mixing on a magnetic stir plate | 2 |
| 3. | Autoclaved liquid agar is measured into petri plates with an automatic pipetting machine | 2 |
| 4. | Air hood (for laminar, sterile air) fitted with oxygen tank to aseptically dispense a suspension of spores | 3 |
| 5. | An atomized suspension of spores is blown onto solidified agar medium | 3 |
| 6. | Details of air-mist applicator construction | 4 |
| 7. | Agar plates with fungal inoculum are maintained on racks in the incubation room | 4 |
| 8. | Spore-collection apparatus attached to collection jar containing spores | 5 |
| 9. | Brush is used to sweep spores into piles for collection | 5 |
| 10. | Suction orifice vacuums spores through hose into the collection jar | 5 |
| 11. | Before refrigerator storage, spores are dried with silica-gel crystals in airtight desiccating jar | 6 |


## TABLES


| | | |
|---|---|---|
| 1. | Cost of labor and expendable items specifically purchased for laboratory spore production (1978–79) | 6 |
| 2. | Frequency distribution of $LC_{50}$ values (for *T. ni* larvae) and spore counts of 31 production batches of *Nomuraea rileyi* spores | 6 |

# Methods and Costs of Producing *Nomuraea rileyi* Conidiospores

By James V. Bell,[1] Robert J. Hamalle,[2] and Carlo M. Ignoffo[3]

## ABSTRACT

This paper presents details for mass propagation of the insect mycopathogen *Nomuraea rileyi* (Farlow) Samson and the estimated cost of conidiospore production. Under sterile conditions, spores were seeded in petri plates that contained a medium of Sabouraud's maltose agar and yeast extract. The cultures were maintained at 23°±2° C and 40%–70% relative humidity. After a 21-day incubation period, spores were vacuum-collected, desiccated with silica-gel, and stored at 4° C. The estimated cost of producing 5.5 kg of spore powder in 1978–79 ($1.75/g) was based on salaries, expendable materials, and the estimated utility charges. Laboratory bioassays of spore activity, spore number, and bacterial contamination were evaluated for each production batch of spores. Index terms: *Nomuraea rileyi* (Farlow) Samson, insect mycopathogens, spore bioassays, spore-production costs.

## INTRODUCTION

Many lepidopterous pests of agricultural crops are susceptible to the insect mycopathogen *Nomuraea rileyi* (Farlow) Samson. This fungus is known to cause natural outbreaks of disease in epizootic proportions during seasons with favorable weather in several pest species, including the cabbage looper, *Trichoplusia ni* (Hübner); green cloverworm, *Plathypena scabra* (Fabricius); velvetbean caterpillar, *Anticarsia gemmatalis* Hübner; and other lepidopterous insects (Getzin 1961, Ignoffo et al. 1976a, 1976b). Although production methods for conidiospores have been reported (Bell 1975), further studies on mass-production methods and costs are important features for successful exploitation of the fungus in insect pest management programs.

In 1978, a cooperative program between researchers at U.S. Agricultural Research Service facilities at Stoneville, Miss., and Columbia, Mo., was established to produce about 5 kg of unadulterated *N. rileyi* spore powder for use in large-scale field testing against lepidopterous pests in soybeans. This program provided an opportunity to evaluate mass-production methods and to estimate the costs for spore production.

## SPORE PRODUCTION

### Method

The spore seeding stock for mass production originated with a Stoneville strain of *N. rileyi*

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FIGURE 1.—Weighing Sabouraud's maltose agar powder medium.

FIGURE 2.—Agar powder distributed into carboys for heating and mixing on a magnetic stir plate.

isolated from field-collected corn earworms, *Heliothis zea* (Boddie), and was subcultured in vitro three times. The dehydrated medium consisted of Difco Sabouraud's (Difco Laboratories, Detroit, Mich.) maltose agar with the addition of 1% yeast extract and 1% agar, and these were proportionately weighed on a torsion balance (fig. 1). The dry ingredients were funneled into 8,000-ml Kimax (Owens-Illinois, Inc., Toledo, Ohio) tubulation carboys containing 4,500 ml of distilled water and a magnetic stir bar. These aqueous mixtures were agitated and heated on magnetic stir plates until all materials blended into a homogenous fluid (fig. 2). The carboys were capped with aluminum foil and the contents sterilized in a Castle (Wilmot Castle Co., Rochester, N.Y.) Thermatic-60 autoclave at 15 lb/in$^2$ for one-half hour. After the autoclaving, the carboys with the hot agar medium were elevated on the stir plates and attached with rubber tubing to a Brewer (Fisher Scientific Co., Pittsburgh, Pa.) automatic pipetting machine that dispensed 80 ml of the medium into 150- by 15-mm sterile plastic disposable petri plates (fig. 3).

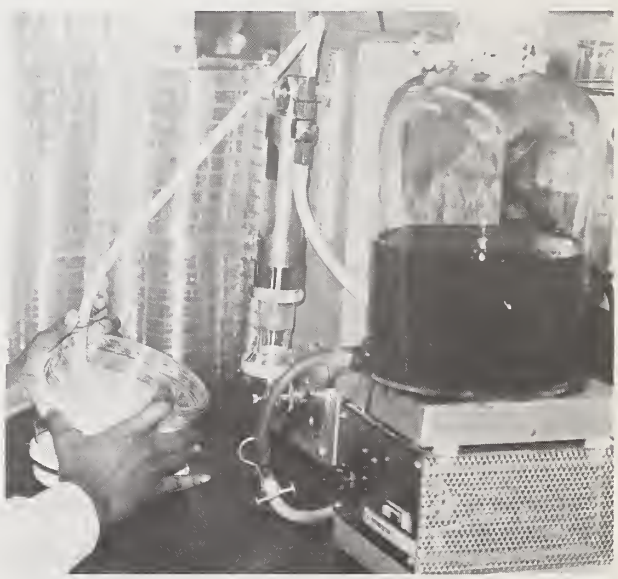

FIGURE 3.—Autoclaved liquid agar is measured into petri plates with an automatic pipetting machine.

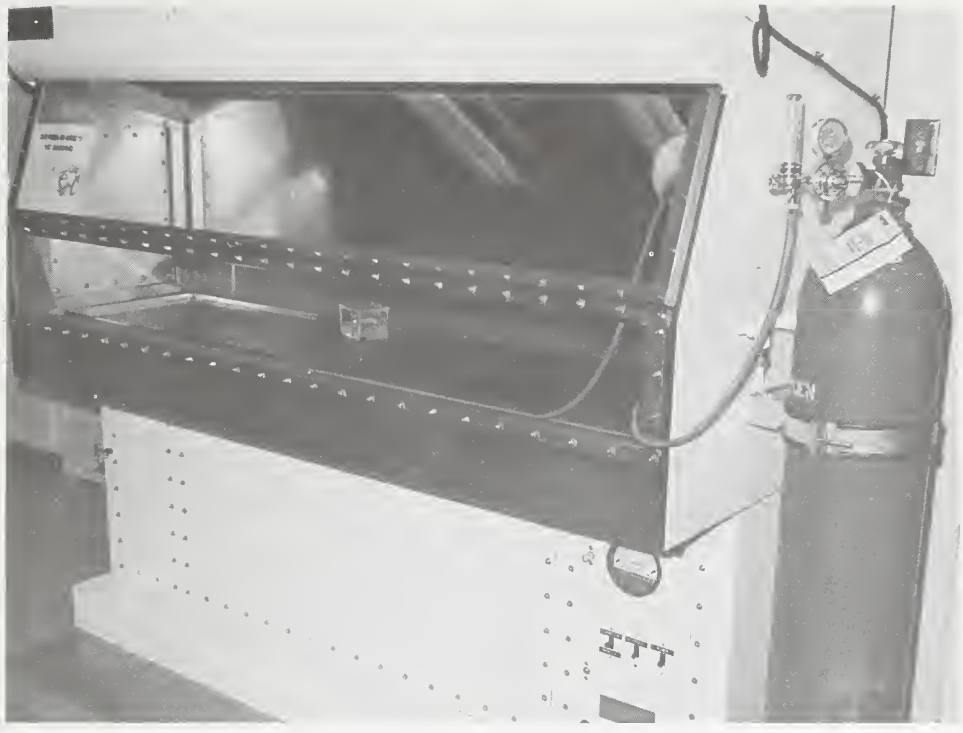


FIGURE 4.—Air hood (for laminar, sterile air) fitted with oxygen tank to aseptically dispense a suspension of spores.

FIGURE 5.—An atomized suspension of spores is blown onto solidified agar medium.

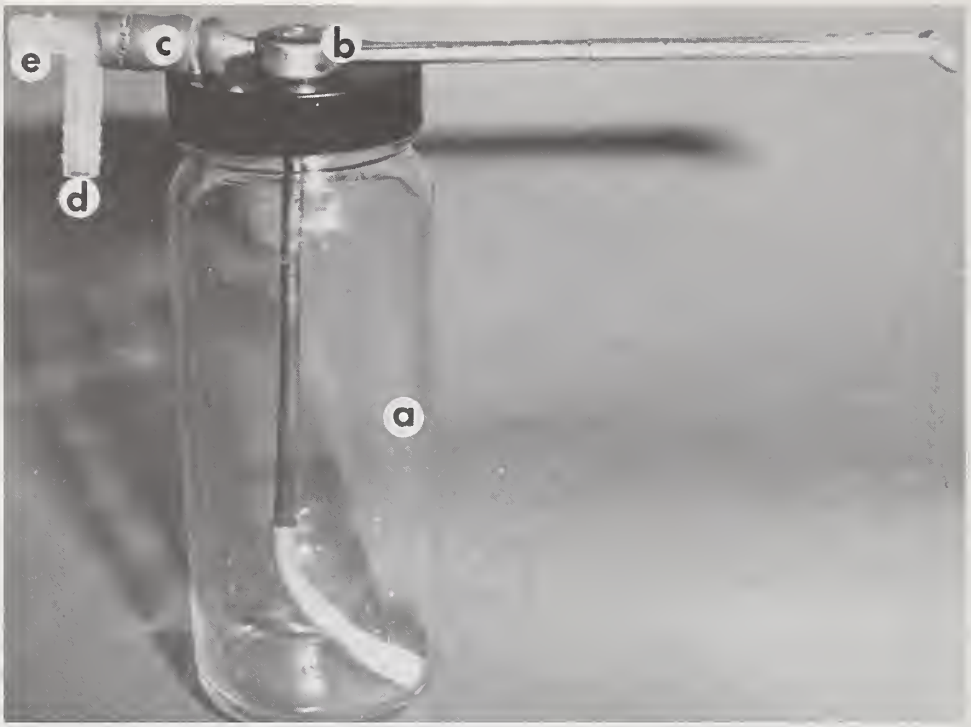


FIGURE 6.—Details of air-mist applicator construction; 8-oz screwcap bottle (*a*) has the lid bored and threaded for the metal atomizer (*b*). The short piece of rubber tubing (*c*) is wired to a plastic T-joint. The rubber tubing from the oxygen tank is attached to the bottom of the T (*d*); when the operator's thumb is applied to the open end (*e*), the air-pressure system is enclosed, and the sprayer tip discharges an aqueous burst of suspended spores.

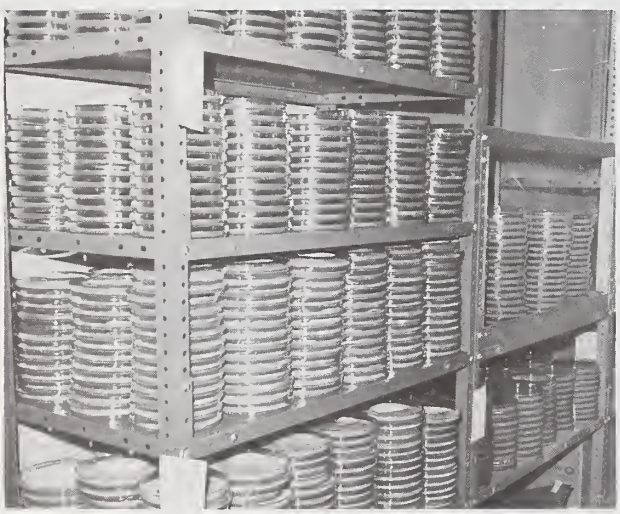

FIGURE 7.—Agar plates with fungal inoculum are maintained on racks in the incubation room.

Seeding of the agar plates with spores was conducted under an air hood providing sterile air (Baker Co., Inc., Sanford, Maine), and medical-grade oxygen for air pressure was filtered through a Gelman (Gelman Instrument Co., Ann Arbor, Mich.) pleated-membrane capsule with a 0.2-$\mu$m microbial-retention capability (fig. 4). This system supplied the necessary air pressure to deliver the desired spore inoculum of about 0.3 ml ($105\times10^6$ spores/ml) onto each agar culture plate without including contaminants usually found in ordinary air-pressure systems (fig. 5). The air-mist applicator is shown in figure 6.

The weekly spore-production flow consisted of about 600 disposable petri plates, each containing 80 ml of agar. These spore-seeded plates were stacked in the incubation room on racks (fig. 7) and were maintained during the 21-day growth and spore-production period at 23°±2° C and 40%–70% relative humidity. The only light the cultures received was overhead fluorescent lighting during the standard 8-hour work schedule, 5 days a week.

FIGURE 8.—Spore-collection apparatus attached to collection jar containing spores.

FIGURE 9.—Brush is used to sweep spores into piles for collection.

FIGURE 10.—Suction orifice vacuums spores through hose into the collection jar.

## YIELD

The continual 21-day production method consisted of seeding new cultures and harvesting spores each week. After 5.5 months, a total production of 5,695 g, or about 5.7 kg, of unadulterated spore powder had been harvested. Assembly of the apparatus for spore collection from culture plates of agar (fig. 8) is detailed by Hamalle and Bell (1976). Two rubber hoses were connected to glass tubing that entered the top of the filtration unit. One hose was attached to a vacuum line and the other was connected to a glass-tubing wand that was terminated with a brush on one side and a suction orifice on the other. The operator, working under an exhaust hood and wearing a face mask, brushed spores into piles on the agar surface (fig. 9), and then turned the wand to the suction orifice and vacuumed the spores into the lower collecting jar (fig. 10).

Final spore treatment consisted of drying the unadulterated spore mass in a glass desiccator jar that contained a large volume of silica-gel crystals (fig. 11). The desiccator was closed with its lid, and the spores were dried for 7 days, which was sufficient to remove any free water. The spores remained in the preweighed collec-

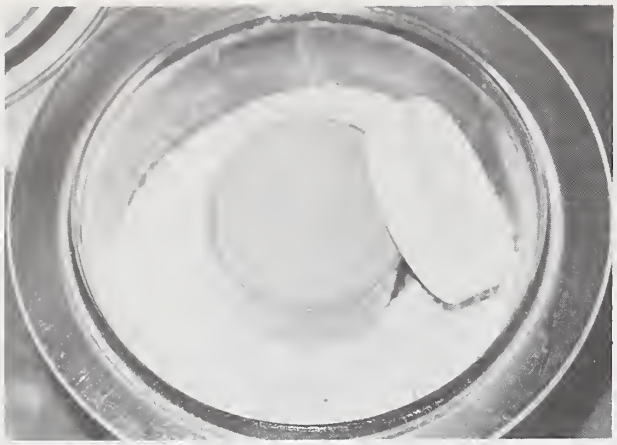

FIGURE 11.—Before refrigerator storage, spores are dried with silica-gel crystals in airtight desiccating jar.

**Table 1.—Cost of labor and expendable items specifically purchased for laboratory spore production (1978-79)**

| Item | Cost |
|---|---|
| One employee, full time | $3,834.49 |
| Disposable petri plates | 2,845.00 |
| Sabouraud's maltose agar medium | 2,987.50 |
| Yeast extract | 50.00 |
| Oxygen and equipment | 95.00 |
| Chemicals and sanitation sprays | 98.90 |
| Subtotal | 9,910.89 |
| Miscellaneous estimated charges for electricity, gas, water, etc. | 64.00 |
| Total | 9,974.89 |

**Table 2.—Frequency distribution of $LC_{50}$ values (for *T. ni* larvae) and spore counts of 31 production batches of *Nomuraea rileyi* spores**

| $LC_{50}$ | | Spore counts | |
|---|---|---|---|
| Spores/mm² | Percentage of sample | No. $\times 10^9$ (grams) | Percentage of sample |
| <0.1 | 3.2 | 1-10 | 19.4 |
| 0.1-5.0 | 41.9 | 21-50 | 0.0 |
| 5.1-10.0 | 35.5 | 51-60 | 3.2 |
| 10.1-15.0 | 3.2 | 61-70 | 6.4 |
| 15.1-20.0 | 3.2 | 71-80 | 6.4 |
| 20.1-25.0 | 3.2 | 81-90 | 35.5 |
| 25.1-30.0 | 3.2 | 91-100 | 9.7 |
| 30.1-35.0 | 0.0 | 101-110 | 12.9 |
| 35.1-40.0 | 3.2 | 111-120 | 6.4 |
| >40.00 | 3.2 | | |

tion jar, the total dry spore weight was established, and the materials were stored at 5° C for later determination of spore counts, biological activity, and bacterial contamination.

### Cost

The cost of spore production was calculated on actual costs for goods and services and estimated costs for such necessities as water, gas, and electricity (table 1). The final cost estimate for 5,695 g was $1.75/g and was based on dates of purchase during times of numerous price increases (1978-79).

## LABORATORY BIOASSAYS

### Spore Counts

Frequency distributions of spore counts and $LC_{50}$ values were established on *Trichoplusia ni* (Hübner) larvae for all 31 production batches (table 2). About one-fifth of the samples had counts ranging from $1\times10^9$ to $10\times10^9$ spores/g; the other four-fifths had counts ranging from $51\times10^9$ to $120\times10^9$ spores/g. The average standard error for the frequency group $1\times10^9$ to $10\times10^9$ was $7.4\times10^9\pm0.6\times10^9$ spores/g; for the frequency group $51\times10^9$ to $120\times10^9$, it was $90.3\times10^9\pm3.9\times10^9$. The average standard error for all 31 samples was $74.2\times10^9\pm6.8\times10^9$/g.

### Biological Activity

A standardized leaflet-surface treatment technique (Ignoffo et al. 1976b) was used to determine the spore activity of all 31 production batches. With this method, twenty 24-hour-old *T. ni* larvae were fed soybean-leaflet disks (18 cm², 4.8-cm-diameter disk). The disks were treated on upper and lower surfaces with serial concentrations of spores dispensed in 0.1-ml sterile distilled water containing 0.05% Tween 80 (Rohm and Haas, Philadelphia, Pa.). The larvae used in the bioassays were from a colony that has been continuously reared on a semisynthetic diet since 1959 (Ignoffo 1963).

Bioassays were conducted on all production batches, and one batch, selected as a standard, was repeatedly bioassayed (5 replicates) at rates of 0, 3, 10, 30, and 100 spores/mm². The rate-mortality regression equation ($y=a+bx$) cal-

culated for this standard was $y=2.890+1.772x$ ($y$=the percent mortality expressed in probit, and $x$=the spores/mm$^2$ expressed in log). The $LC_{50}$ standard error of the mean for the standard was 16.8±4.0 spores/mm$^2$; the standard error of the slope (1.722) of the regression line was ±0.152. Each production batch was bioassayed once with the same rates as the standard, and the $LC_{50}$ was calculated with the regression equation. Of the 31 samples, one batch had no activity and one batch had an $LC_{50}$ of 320 spores/mm$^2$. Most batches, however, had an $LC_{50}$ between 0.1 to 5.0 spores/mm$^2$ (table 2).

### Microbial Contamination

Because bacterial contamination could hamper production greatly, total viable-bacterial-cell counts were made on 15 of the 31 production batches of spores. A 100-mg subsample of spores from each batch was suspended in 10 ml of sterile distilled water and then decimally diluted three times. A 0.1-ml and 1.0-ml aliquot of each dilution was then dispensed in petri plates, and to this was added about 15 ml of tryptic-soy-agar. The spores and agar were thoroughly mixed; and, when the agar solidified, the plates were incubated at 30°±1° C. Viable cells (colonies) were counted 18–20 hours later. Bacteria were detected in only 1 of the 15 production batches tested. The number of total viable bacterial cells/g for the contaminated sample averaged 18.9±3.0 cells/mg or the equivalent of $4\times10^6$ spores for each bacterial cell.

## DISCUSSION

Under the conditions of this spore-production method, an exact figure for all expenditures was impossible. Most laboratory equipment was previously purchased and had been in use for other research assignments. The utility charges could not be accurately prorated from the total cost for the entire building. The method we used to estimate electrical costs involved calculation of a watts-per-hour basis for electrical equipment used at the rate charges for the building. The expendable items and labor costs were calculated for the time period involved.

Several questions enter into any discussion about whether a spore-production process such as this has future economic potential. The following questions should be considered: (1) Can quantity production be realized? (2) Can a standardized spore product be consistently produced and stored for effective insect control? (3) Is this product cheap to produce commercially? (4) Is this product more efficacious than materials now available on the market for insect pest management? This study has considered the first two questions with some success; however, further refinements are necessary. The third and fourth considerations await future research and development.

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